CLAIMS

We claim:

1. A method of amplifying messenger RNA, the method comprising

(a) mixing one or more RT primers with a nucleic acid sample and reverse transcribing to produce cDNA strands each comprising one of the RT primers, wherein each RT primer comprises a reverse transcription primer portion,

(b) mixing the cDNA strands with a set of capture probes under conditions that promote hybridization of the cDNA strands to the capture probes,

(c) mixing one or more rolling circle replication primers with the cDNA strands under conditions that promote association of the cDNA strands with the rolling circle replication primers, wherein the rolling circle replication primers each comprise a capture tag, and wherein the association occurs via the capture tag,

(d) mixing one or more amplification target circles with the rolling circle replication primers under conditions that promote association of the rolling circle replication primers with the amplification target circles,

(e) incubating the amplification target circles under conditions that promote replication of the amplification target circles,

wherein replication of the amplification target circles results in the formation of tandem sequence DNA.

2. The method of claim 1 wherein the capture tag associates with the RT primer.

3. The method of claim 1 wherein the reverse transcription primer portion of each RT primer comprises poly T.

4. The method of claim 1 wherein the capture probes are immobilized on a substrate.

5. The method of claim 4 wherein the capture probes are in an array.

6. The method of claim 4 wherein the capture probes are immobilized via a capture tag coupled to the capture probes.

7. The method of claim 1 wherein each capture probe comprises a sequence matching all or a portion of the sequence of messenger RNA molecules of interest.

8. The method of claim 7 wherein the set of capture probes collectively comprise sequence matching all or a portion of the sequence of a plurality of different messenger RNA molecules of interest.

9. The method of claim 8 wherein the plurality of different messenger RNA molecules of interest comprise a set of messenger RNA molecules derived from, or present in, cells from a source of interest.

10. The method of claim 9 wherein the plurality of different messenger RNA molecules are associated with a condition or disease state of the cells or the source of interest.

11. The method of claim 8 wherein the plurality of different messenger RNA molecules of interest comprise a set of messenger RNA molecules representing a catalog of messenger RNA molecules from a source of interest.

12. The method of claim 8 wherein the plurality of different messenger RNA molecules of interest comprise a set of messenger RNA molecules from a plurality of sources of interest.

13. The method of claim 1 wherein the ends of the capture probes are extendable when a cDNA strand is hybridized to the capture probe.

14. The method of claim 13 wherein the ends of the capture probes are designed to be extendable only when a cDNA strand corresponding to a particular form of a messenger RNA of interest is hybridized to the capture probe.

15. The method of claim 1 wherein the ends of the capture probes are not extendable by polymerase.

16. The method of claim 1 further comprising, prior to step (c),

mixing one or more half probes with the cDNA strands, wherein each half probe is designed to hybridize to a cDNA strand adjacent to where a capture probe hybridizes,

ligating half probes and capture probes hybridized to cDNA strands.

17. The method of claim 16 further comprising, following ligation,

incubating the capture probes at a temperature above the melting temperature of the capture probe but below the melting temperature of the ligated capture probe/half probe.

18. The method of claim 1 further comprising, simultaneous with, or following, step (d),

mixing a secondary DNA strand displacement primer with the amplification target circles and incubating under conditions that promote hybridization between the tandem sequence DNA and the secondary DNA strand displacement primer and replication of the tandem sequence DNA,

wherein replication of the tandem sequence DNA results in the formation of secondary tandem sequence DNA.

19. The method of claim 18 further comprising, simultaneous with step (e),

mixing a tertiary DNA strand displacement primer with the amplification target circles.

20. The method of claim 1 further comprising detecting the tandem sequence DNA, wherein detection of tandem sequence DNA indicates that the corresponding messenger RNA molecule was present in the nucleic acid sample.

21. The method of claim 20 wherein the tandem sequence DNA is detected while in association with the capture probes.

22. The method of claim 21 wherein the identity of the capture probe associated with a tandem sequence DNA indicates the identity of the corresponding messenger RNA molecule.

23. The method of claim 21 wherein the tandem sequence DNA is detected at the site where the capture probe is located, and wherein the location of the capture probe indicates the identity of the corresponding messenger RNA molecule.

24. The method of claim 20 wherein detection is mediated by detection probes or by a detection label incorporated in the tandem sequence DNA.

25. The method of claim 24 wherein the detection label is a ligand.

26. The method of claim 25 wherein the ligand is biotin or BrdU.

27. The method of claim 26 wherein the ligand is BrdU, wherein the tandem sequence DNA is detected by associating an anti-BrdU antibody with the tandem sequence DNA and detecting the anti-BrdU antibody.

28. The method of claim 27 wherein the anti-BrdU antibody comprises a label, wherein the anti-BrdU antibody is detected by detecting the label.

29. The method of claim 28 wherein the label on the anti-BrdU antibody is a fluorophore.

30. The method of claim 28 wherein the fluorophore is phytoerythrin.

31. The method of claim 20 further comprising

mixing a set of detection probes with the tandem sequence DNA under conditions that promote hybridization between the tandem sequence DNA and the detection probes, and

detecting a plurality of different sequences present in the tandem sequence DNA.

32. The method of claim 1 wherein the tandem sequence DNA is collapsed using collapsing probes.

33. The method of claim 32 wherein at least one of the collapsing probes is a collapsing detection probe.

34. The method of claim 32 wherein the tandem sequence DNA is collapsed by mixing the collapsing probes with the tandem sequence DNA, and incubating under conditions that promote hybridization between the collapsing probes and the tandem sequence DNA.

35. The method of claim 34 further comprising, prior to or simultaneous with the mixing of the collapsing probes with the tandem sequence DNA, mixing detection probes with the tandem sequence DNA, and incubating under conditions that promote hybridization between the detection probes and the tandem sequence DNA.

36. The method of claim 32 wherein the collapsing probes comprise ligands, haptens, or both coupled to or incorporated into oligonucleotides.

37. The method of claim 1 wherein the RT primer comprises a capture tag.

38. The method of claim 37 wherein the capture tag on the RT primer is selected from the group consisting of biotin, digoxigenin, bromodeoxyuridine, or other hapten.

39. The method of claim 37 wherein the cDNA strands comprise capture tags.

40. The method of claim 1 wherein the cDNA strands comprise capture tags.

41. The method of claim 40 wherein the capture tags on the cDNA strands are selected from the group consisting of biotin, digoxigenin, bromodeoxyuridine, or other hapten.

42. The method of claim 1 wherein the association is covalent.

43. The method of claim 1 wherein the association is non-covalent.

44. The method of claim 43 wherein the association occurs between a protein and a nucleic acid.

45. The method of claim 44 wherein the association occurs between two proteins.

46. The method of claim 41 wherein the capture tags on the cDNA strands are biotin.

47. The method of claim 46 wherein the capture tags on the rolling circle replication primers comprise antibodies that bind biotin.

48. A method of amplifying messenger RNA, the method comprising

(a) mixing one or more RT primers with a nucleic acid sample and reverse transcribing to produce cDNA strands each comprising one of the RT primers,

(b) fragmenting the cDNA strands to form fragmented cDNA,

(c) adding a capture tag to the fragmented cDNA,

(d) mixing the fragmented cDNA with a set of capture probes under conditions that promote hybridization of the fragmented cDNA to the capture probes,

(e) mixing one or more rolling circle replication primers with the fragmented cDNA under conditions that promote association of the fragmented cDNA with the rolling circle replication primers, and wherein the association occurs via the capture tag,

(f) mixing one or more amplification target circles with the rolling circle replication primers under conditions that promote association of the rolling circle replication primers with the amplification target circles,

(g) incubating the amplification target circles under conditions that promote replication of the amplification target circles,

wherein replication of the amplification target circles results in the formation of tandem sequence DNA.

49. The method of claim 48 wherein the rolling circle replication primers each comprise a capture tag.

50. The method of claim 49 wherein association of the rolling circle replication primers with the cDNA occurs via association of the capture tag added to the fragmented cDNA and the capture tag in the rolling circle replication primers.

51. The method of claim 48 wherein the capture tag is added to the fragmented cDNA by terminal transferase.

52. The method of claim 51 wherein the capture tag is biotinylated-ddNTP.

53. A method of amplifying messenger RNA, the method comprising

(a) mixing one or more RT primers with a nucleic acid sample and reverse transcribing to produce cDNA strands each comprising one of the RT primers, wherein each RT primer comprises a reverse transcription primer portion and a capture tag,

(b) mixing the cDNA strands with a set of capture probes under conditions that promote hybridization of the cDNA strands to the capture probes,

(c) mixing one or more rolling circle replication primers with the cDNA strands under conditions that promote association of the cDNA strands to the rolling circle replication primers, and wherein the association occurs through the capture tag,

(d) mixing one or more amplification target circles with the rolling circle replication primers under conditions that promote association of the rolling circle replication primers with the amplification target circles,

(e) incubating the amplification target circles under conditions that promote replication of the amplification target circles,

wherein replication of the amplification target circles results in the formation of tandem sequence DNA.

54. The method of claim 53 wherein the rolling circle replication primers each comprise a capture tag.

55. The method of claim 54 wherein association of the rolling circle replication primers with the cDNA occurs via association of the capture tag added to the cDNA and the capture tag in the rolling circle replication primers.

56. A method of amplifying messenger RNA, the method comprising

(a) mixing one or more RT primers with a nucleic acid sample and reverse transcribing to produce cDNA strands each comprising one of the RT primers, wherein each RT primer comprises a reverse transcription primer portion, wherein the cDNA comprises a capture tag,

(b) mixing the cDNA strands with a set of capture probes under conditions that promote hybridization of the cDNA strands to the capture probes,

(c) mixing one or more rolling circle replication primers with the cDNA strands under conditions that promote association of the cDNA strands with the rolling circle replication primers, and wherein the association occurs through the capture tag,

(d) mixing one or more amplification target circles with the rolling circle replication primers under conditions that promote association of the rolling circle replication primers with the amplification target circles,

(e) incubating the amplification target circles under conditions that promote replication of the amplification target circles,

wherein replication of the amplification target circles results in the formation of tandem sequence DNA.

57. The method of claim 56 wherein the rolling circle replication primers each comprise a capture tag.

58. The method of claim 57 wherein association of the rolling circle replication primers with the cDNA occurs via association of the capture tag incorporated into the cDNA and the capture tag in the rolling circle replication primers.

59. The method of 56 wherein the capture tag is derived from allyl amine dUTP.

60. The method of 59 wherein the amplification target circle hybridizes with a rolling circle amplification primer comprising an NHS ester.

61. The method of claim 57 wherein the capture tag is derived from incorporation of biotinylated-ddNTP into the cDNA.

62. A method of amplifying messenger RNA, the method comprising

(a) mixing one or more RT primers with a nucleic acid sample and reverse transcribing to produce cDNA strands each comprising one of the RT primers, wherein each RT primer comprises a reverse transcription primer portion and a rolling circle replication primer portion,

wherein the reverse transcription primer portion and the rolling circle replication primer portion each comprise a 5' end, wherein the reverse transcription primer portion and the rolling circle replication primer portion are not linked via their 5' ends,

(b) mixing the cDNA strands with a set of capture probes under conditions that promote hybridization of the cDNA strands to the capture probes,

(c) mixing one or more amplification target circles with the rolling circle replication primer portions under conditions that promote association of the rolling circle replication primer portions with the amplification target circles,

(d) incubating the amplification target circles under conditions that promote replication of the amplification target circles,

wherein replication of the amplification target circles results in the formation of tandem sequence DNA.

63. A kit for amplifying messenger RNA, the kit comprising,

(a) one or more amplification target circles,

wherein the amplification target circles each comprise a single-stranded, circular DNA molecule comprising a primer complement portion, and

(b) one or more RT primers,

wherein the RT primers each comprise a reverse transcription primer portion and a rolling circle replication primer portion,

wherein the reverse transcription primer portion and the rolling circle replication primer portion each comprise a 5' end, wherein the reverse transcription primer portion and the rolling circle replication primer portion are not linked via their 5' ends, wherein both the reverse transcription primer portion and the rolling circle replication primer portion can prime nucleic acid replication,

wherein the rolling circle replication primer portion is complementary to a portion of one or more amplification target circles, and

(c) one or more capture probes,

wherein each capture probe comprises a sequence matching all or a portion of the sequence of messenger RNA molecules of interest.

64. The kit of claim 63 wherein the reverse transcription primer portion of each RT primer comprises poly T.

65. The kit of claim 63 further comprising a secondary DNA strand displacement primer comprising a single-stranded, linear nucleic acid molecule comprising a matching portion that matches a portion of one or more of the amplification target circles.

66. The kit of claim 65 further comprising a tertiary DNA strand displacement primer comprising a single-stranded, linear nucleic acid molecule comprising a

complementary portion that is complementary to a portion of one or more of the amplification target circles.

67. A mixture comprising

(a) cDNA strands produced by incubating one or more RT primers with a nucleic acid sample and reverse transcribing, wherein each cDNA strand comprises one of the RT primers, wherein each RT primer comprises a reverse transcription primer portion,

(b) a set of capture probes hybridized to the cDNA strands,

(c) one or more rolling circle replication primers associated with the cDNA strands, wherein the rolling circle replication primers each comprise a capture tag, and wherein the association occurs via the capture tag,

(d) one or more amplification target circles associated with the rolling circle replication primers.

68. A method of using messenger RNA, the method comprising

replicating one or more amplification target circles to produce one or more tandem sequence DNAs,

wherein each tandem sequence DNA is coupled to a rolling circle replication primer, wherein the rolling circle replication primer is associated with a cDNA strand, wherein the rolling circle replication primer comprises a capture tag, wherein the association occurs via the capture tag, wherein the cDNA strand is hybridized to a capture probe, wherein the cDNA strand comprises an RT primer, wherein the cDNA strand is produced by reverse transcribing a nucleic acid sample with the RT primer.

69. A method of using messenger RNA, the method comprising

replicating one or more amplification target circles to produce one or more tandem sequence DNAs,

wherein each tandem sequence DNA is coupled to a rolling circle replication primer, wherein the rolling circle replication primer is associated with a fragmented cDNA strand, wherein the fragmented cDNA strand is hybridized to a capture probe, wherein the fragmented cDNA comprises a capture tag, wherein the association occurs via the capture tag, wherein the fragmented cDNA strand is a fragment of a cDNA strand, wherein the cDNA strand comprises an RT primer, wherein the cDNA strand is produced by reverse transcribing a nucleic acid sample with the RT primer.

70. A method of using messenger RNA, the method comprising

replicating one or more amplification target circles to produce one or more tandem sequence DNAs,

wherein each tandem sequence DNA is coupled to a rolling circle replication primer, wherein the rolling circle replication primer is associated with a cDNA strand, wherein the cDNA strand is hybridized to a capture probe, wherein the cDNA strand comprises an RT primer, wherein the RT primer comprises a capture tag, wherein the association occurs via the capture tag, wherein the cDNA strand is produced by reverse transcribing a nucleic acid sample with the RT primer.

71. A method of using messenger RNA, the method comprising

replicating one or more amplification target circles to produce one or more tandem sequence DNAs,

wherein each tandem sequence DNA is coupled to a rolling circle replication primer, wherein the rolling circle replication primer is associated with a cDNA strand, wherein the cDNA strand comprises a capture tag, wherein the association occurs via the capture tag, wherein the cDNA strand is hybridized to a capture probe, wherein the cDNA strand comprises an RT primer, wherein the cDNA strand is produced by reverse transcribing a nucleic acid sample with the RT primer.

72. A method of using messenger RNA, the method comprising

replicating one or more amplification target circles to produce one or more tandem sequence DNAs,

wherein each tandem sequence DNA is coupled to a rolling circle replication primer portion of an RT primer that comprises the rolling circle replication primer portion and a reverse transcription primer portion, wherein the cDNA strand is hybridized to a capture probe, wherein the cDNA strand comprises the RT primer, wherein the cDNA strand is produced by reverse transcribing a nucleic acid sample with the RT primer,

wherein the reverse transcription primer portion and the rolling circle replication primer portion each comprise a 5' end, wherein the reverse transcription primer portion and the rolling circle replication primer portion are not linked via their 5' ends.

73. A method of amplifying messenger RNA, the method comprising

(a) mixing one or more RT primers with a nucleic acid sample and reverse transcribing to produce cDNA strands each comprising one of the RT primers, wherein each RT primer comprises a reverse transcription primer portion, wherein the cDNA strands comprise capture tags, wherein the capture tags on the cDNA strands are biotin,

(b) mixing the cDNA strands with a set of capture probes under conditions that promote hybridization of the cDNA strands to the capture probes,

(c) mixing one or more rolling circle replication primers with the cDNA strands under conditions that promote association of the cDNA strands with the rolling circle replication primers, wherein the rolling circle replication primers each comprise a capture tag, wherein the capture tags on the rolling circle replication primers comprise antibodies that bind biotin, wherein the association occurs via the capture tags on the cDNA strands and the capture tags on the rolling circle replication primers,

(d) mixing one or more amplification target circles with the rolling circle replication primers under conditions that promote association of the rolling circle replication primers with the amplification target circles,

(e) incubating the amplification target circles under conditions that promote replication of the amplification target circles,

wherein replication of the amplification target circles results in the formation of tandem sequence DNA,

(f) detecting the tandem sequence DNA, wherein detection of tandem sequence DNA indicates that the corresponding messenger RNA molecule was present in the nucleic acid sample,

wherein detection is mediated by a detection label incorporated in the tandem sequence DNA, wherein the detection label is BrdU,

wherein the tandem sequence DNA is detected by associating an anti-BrdU antibody with the tandem sequence DNA and detecting the anti-BrdU antibody,

wherein the anti-BrdU antibody comprises a label, wherein the label is phytoerythrin, wherein the anti-BrdU antibody is detected by detecting the label.